Amyloid- β -Induced Action Potential Desynchronization and Degradation of Hippocampal Gamma Oscillations Is Prevented by Interference with Peptide Conformation Change and Aggregation

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The amyloid- β hypothesis of Alzheimer's Disease (AD) focuses on accumulation of amyloid- β peptide (A β) as the main culprit for the myriad physiological changes seen during development and progression of AD including desynchronization of neuronal action potentials, consequent development of aberrant brain rhythms relevant for cognition, and final emergence of cognitive deficits.

The aim of this study was to elucidate the cellular and synaptic mechanisms underlying the A β -induced degradation of gamma oscillations in AD, to identify aggregation state(s) of A β that mediate the peptides neurotoxicity, and to test ways to prevent the neurotoxic A β effect.

We show that A β_{1-42} in physiological concentrations acutely degrades mouse hippocampal gamma oscillations in a concentration- and time-dependent manner. The underlying cause is an A β -induced desynchronization of action potential generation in pyramidal cells and a shift of the excitatory/inhibitory equilibrium in the hippocampal network. Using purified preparations containing different aggregation states of A β , as well as a designed ligand and a BRICHOS chaperone domain, we provide evidence that the severity of A β neurotoxicity increases with increasing concentration of fibrillar over monomeric A β forms, and that A β -induced degradation of gamma oscillations and excitatory/inhibitory equilibrium is prevented by compounds that interfere with A β aggregation.

Our study provides correlative evidence for a link between A β -induced effects on synaptic currents and AD-relevant neuronal network oscillations, identifies the responsible aggregation state of A β and proofs that strategies preventing peptide aggregation are able to prevent the deleterious action of A β on the excitatory/inhibitory equilibrium and on the gamma rhythm.

Key words: Alzheimer's disease; amyloid- β peptide; BRICHOS domain; gamma oscillations; hippocampus; neuronal synchronization

Introduction

Rhythmic electrical activity or oscillations are a crucial component of brain function (Sederberg et al., 2007). Oscillations in the gamma-frequency band (30–80 Hz) bind neurons into a common temporal matrix, enabling precise temporal coding, spike

timing-dependent plasticity, and formation of neuronal assemblies (Singer, 1993). Gamma oscillations are dependent on precise timing of action potential generation and the finely balanced interplay between excitatory and inhibitory neurotransmission in the neuronal network. Gamma oscillations have been suggested to underlie higher cognitive functions, such as sensory perception, attention, and memory (Singer, 1993), and are known to be significantly degraded in Alzheimer Disease (AD) patients, who suffer from deficiencies in their cognitive faculties (Ribary et al., 1991; Uhlhaas and Singer, 2006; Jelic and Kowalski, 2009). It has been shown that increased A β levels in AD mouse models result in disrupted timing of evoked action potentials (APs; Stern et al., 2004). However, the exact cellular mechanisms underlying the AD-related degradation of oscillatory activity are yet to be elucidated.

The amyloid cascade hypothesis of AD pathogenesis focuses on toxic amyloid β -peptide (A β) aggregates as the main culprit for the deleterious effect on brain function observed in AD and

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Figure 1. Effects of A β on hippocampal gamma oscillations. **A**, Example traces and example power spectra of control gamma oscillations and their degradation by increasing concentrations of A β . **B**, Summary box plot of gamma oscillation degradation by increasing concentrations of A β . **C**, Example traces and example power spectra of control gamma oscillations and their degradation by increasing exposure times to 50 nM A β . **D**, Summary box plot of gamma oscillation degradation by increasing exposure times to 1 μ M A β and 50 nM A β (inset).

A β has been suggested to be responsible for early cognitive decline in AD (Mucke et al., 2000; Selkoe, 2002; Walsh and Selkoe, 2007). Overall, many features of AD pathology can be observed in mutant mice overexpressing amyloid precursor protein (APP) and displaying increased levels of A β (Games et al., 1995; Mucke et al., 2000; Götz et al., 2004). Recent studies point to impaired gamma oscillations paralleled by an epileptic phenotype in an AD mouse model, a pathology suggested to be based on a deficit in inhibitory activity (Verret et al., 2012). Many neuronal AD pathologies were also replicated in wild-type rodent brain slices with acute application of A β , including synaptic LTP impairment (Shemer et al., 2006; Shankar et al., 2008), as well as neuronal hyperexcitability and resulting network epileptiform activity (Palop et al., 2007; Minkeviciene et al., 2009; Zilberter et al., 2013). Other recent studies also show that A β is capable of degrading neuronal gamma oscillations, potentially through similar mechanisms (Nerelius et al., 2009; Pena-Ortega et al., 2012; for review, see Pena-Ortega, 2013).

According to the amyloid cascade hypothesis, processing of APP produces A β , aggregation, and fibril formation of which give rise to synaptotoxicity (Mucke et al., 2000) followed by network dysfunction, and eventually dementia (Hardy and Selkoe, 2002; Palop et al., 2006; Palop and Mucke, 2010; Mucke and Selkoe, 2012). Many studies indicate that prefibrillar intermediates, including small soluble oligomers, are most potent in causing neuronal dysfunction, suggesting that oligomeric intermediates in

the aggregation process are more toxic than fibrils (Hardy and Selkoe, 2002; Shankar et al., 2008). However, A β fibrils are apparently toxic in cell cultures (Chimon et al., 2007; Qiang et al., 2012). Recently, it was shown that secondary nucleation into oligomeric species is mediated by A β monomers that bind to the surface of fibrils, and that this is a major source of toxic species (Cohen et al., 2013). Fibrillar A β may be toxic as such, but may also cause toxicity by nucleating formation of oligomers, provided that monomeric A β is present.

In this study we investigate the relationship between A β -induced degradation of gamma oscillations and the concentration of fibrillar over monomeric A β forms, elucidate the cellular mechanisms responsible for the toxic A β effect and demonstrate the effectiveness of compounds that interfere with A β aggregation in the prevention of A β toxicity.

Materials and Methods

Animals. Experiments were performed in accordance with the ethical permit granted by Norra Stockholms Djurförsöksetiska Nämnd to AF (N45/13). C57BL/6 mice of either sex (postnatal days 14–23, supplied from Charles River Laboratories) were used in all experiments. The animals were deeply anesthetized using isoflurane before being killed by decapitation.

Tissue preparation. The brain was dissected out and placed in ice-cold artificial CSF (ACSF) modified for dissection. This solution contained (in mM): 80 NaCl, 24 NaHCO₃, 25 glucose, 1.25 NaH₂PO₄, 1 ascorbic acid, 3 Na pyruvate, 2.5 KCl, 4 MgCl₂, 0.5 CaCl₂, 75 sucrose. Horizontal

Figure 2. A β desynchronizes PC firing. **A**, Degradation of gamma oscillation power over time by A β . **B**, Gamma oscillation frequency remains unchanged by A β . **C**, AP frequency of PCs is increased by A β . **D**, AP firing phase remains unchanged by A β . **E**, AP firing window increases after exposure to A β .

sections (350 μ m thick) of the ventral hippocampi of both hemispheres were prepared with a Leica VT1200S vibratome (Leica Microsystems). Immediately after slicing sections were transferred to a submerged incubation chamber containing standard ACSF (in mM): 124 NaCl, 30 NaHCO₃, 10 glucose, 1.25 NaH₂PO₄, 3.5 KCl, 1.5 MgCl₂, 1.5 CaCl₂. The chamber was held at 34°C for at least 20 min after dissection. It was subsequently allowed to cool to ambient room temperature ($\sim$ 22°C) for a minimum of 40 min. Peptides were added to the incubation solution 15 min (unless otherwise stated) before transferring slices to the interface-style recording chamber for extracellular recordings or added to the perfusate for patch-clamp recordings in a submerged recording chamber. While incubating, slices were continuously supplied with carbogen gas (5% CO₂, 95% O₂) bubbled into the ACSF.

Electrophysiology. Recordings were performed in hippocampal area CA3 with borosilicate glass microelectrodes, pulled to a resistance of 3–5 M Ω . Local field potentials (LFPs) were recorded in an interface-type chamber (perfusion rate 4.5 ml per min) or a submerged-type chamber (for concomitant LFP/patch-clamp recordings) at 34°C using microelectrodes filled with ACSF placed in stratum pyramidale (Fisahn et al., 1998). LFP gamma oscillations were elicited by applying kainic acid (100 nM) to the extracellular bath (Fisahn et al., 2004). The oscillations were allowed to stabilize for 20 min before any recordings were performed.

The interface chamber recording solution contained the following (in mM): 124 NaCl, 30 NaHCO₃, 10 glucose, 1.25 NaH₂PO₄, 3.5 KCl, 1.5 MgCl₂, 1.5 CaCl₂. The submerged chamber (patch-clamp) recording solution contained the following (in mM): 126 NaCl, 2.5 KCl, 26 NaHCO₃, 1.25 NaH₂PO₄, 1.5 MgCl₂, 1.5 CaCl₂. Action potentials were recorded from pyramidal cells in area CA3 in cell-attached mode with ACSF-containing patch electrodes. The intracellular solution used for patch-clamp recordings of postsynaptic currents contained the following (in mM): CsMeSO₄ 140, HEPES 10, MgCl₂ 2, EGTA 0.6, ATPNa₂ 2, GTPNa₂ 0.3, set to pH 7.2–7.3 with CsOH, osmolality 270–280 mOsm.

Interface chamber LFP recordings were performed with a 4-channel amplifier/signal conditioner M102 amplifier (Electronics laboratory, Faculty of Mathematics and Natural Sciences, University of Cologne, Cologne, Germany). Submerged chamber LFP recordings and patch-clamp recordings were performed using a Multiclamp 700B (Molecular Devices). The signals were sampled at 10 kHz, conditioned using a Hum Bug 50 Hz noise eliminator (LFP signals only; Quest Scientific), software low-pass filtered at 1 kHz, digitized and stored using a Digidata 1322A and Clampex 9.6 software (Molecular Devices).

Analysis. Power spectral density plots (from 60-s-long LFP recordings) were calculated in averaged Fourier-segments of 8192 points using Axograph X (Kagi). Oscillation power was calculated by integrating the power spectral density between 20 and 80 Hz. Patch-clamp recordings and concomitant LFP recordings were analyzed using IGOR Pro (WaveMetrics). Data were binned and analyzed over 1 min periods. For the total quantification, the total values were averaged over a 5 min period after 15 min of A β application compared with the average of a 5 min initial control period. All data presented normalized was normalized to the average of the first 5 min of control recording before A β application.

EPSCs and IPSCs were recorded at -70 mV (EPSCs) and 0 mV (IPSCs) and charge transfer, event amplitude, event frequency, and inter-event-interval (IEI) were analyzed using IGOR Pro with the normalized results representing average values taken over 1 min periods. Paired-pulse ratio experiments were recorded in CA3 pyramidal cells with synaptic potentials evoked by mossy fiber pathway stimulation using a concentric bipolar electrode (dual pulse at 50% maximum response, 100 ms pulse separation, 10 s intersweep interval; values are averages of 10 sweeps). Paired-pulse ratio was defined as the slope of the first EPSP divided by the slope of the second EPSP.

Data are reported as means $\pm$ SEM in the text, and as median and upper/lower quartile in the figure box plots. For statistical analysis the Mann–Whitney U test (two-tailed) was used for unpaired data (extracellular) and Student's t test for paired data (patch-clamp). Mann–Whitney U test results are given as the values of the statistic U , the sample sizes n_1 and n_2 , and the significance value p in Table 1. Significance levels are $*p < 0.05$.

Figure 3. A β alters balance between excitation and inhibition. **A**, In an activated network (100 nM KA), A β increases EPSC charge transfer and decreases IPSC charge transfer. These changes are based on an A β -induced increase of EPSC frequency and amplitude and a decrease of IPSC frequency and amplitude. **B**, In a quiescent network, A β increases EPSC charge transfer

0.05, $^{**}p < 0.01$, $^{***}p < 0.001$. All experiments were performed with parallel controls from the same animal/preparation.

Drugs and chemicals. Chemical compounds used in intracellular and extracellular solutions were obtained from Sigma-Aldrich. Kainic acid was obtained from Tocris Bioscience. The ligand Dec-DETA, designed to stabilize A β in an α -helical conformation, was synthesized as described by Nerelius et al. (2009).

Expression and purification of recombinant proteins. Met-A β ₁₋₄₂ was expressed in *Escherichia coli* BL21 from synthetic genes and purified in batch format using ion exchange and passed through a 30000 Da Vivaspin concentrator filter (Sartorius Stedim Biotech GmbH) to remove large aggregates (Walsh et al., 2009). Purified peptide was concentrated to 50–100 μ M, aliquoted in low-bind Eppendorf tubes (Axygene) and stored at -20°C . The peptide concentration was determined using an extinction coefficient of $1400 \text{ M}^{-1}\text{cm}^{-1}$ at 280 nm. A β ₁₋₄₂ was thawed on ice and diluted to the desired concentration for the respective experiment. Monomeric A β ₁₋₄₂ was obtained by lyophilizing concentrated A β ₁₋₄₂ peptide, dissolving it in 7 M guanidine hydrochloride and subjecting it to a size exclusion chromatography column. Fibrillized A β ₁₋₄₂ was obtained by placing 10 μ M monomeric A β ₁₋₄₂ at 37°C with 500 rpm agitation for 2 h. Thioflavin-T fluorescence was measured to ensure that A β ₁₋₄₂ had fully fibrillized.

Because purified monomeric A β ₁₋₄₂ consists essentially of only one molecule species, its mol-per-volume (molarity) concentration can easily be established. Mixed and fibrillar A β ₁₋₄₂ solutions on the other hand side are of a highly dynamic molecule composition with molarity and number of different molecule species contained in these solutions being essentially stochastic. The molarity of mixed and fibrillar A β ₁₋₄₂ solutions was calculated based on monomeric A β ₁₋₄₂. In general we advocate the use of weight-per-volume concentration instead of mol-per-volume with 50 nM A β ₁₋₄₂ being equal to 226 ng/ml (molecular weight, A β ₁₋₄₂ = 4614 g/mol) because weight-per-volume stays constant, whereas molarity changes during aggregation.

The ProSPC BRICHOS chaperone was made as previously described by Willander et al. (2012). To ensure that ProSPC BRICHOS is free from contaminant, it was subjected to an endotoxin removal column (Thermo Scientific), passed through a 0.22 μ m diameter Millex-GV filter (Millipore) and stored at -20°C .

Spider silk proteins (spidroins) convert from a soluble state dominated by helical and random structures to insoluble fibers during silk formation (Simmons et al., 1996) and amyloid-like fibrils have been found in spider silk (Kenney et al., 2002). We have found that a domain from *Araneus ventricosus* minor ampullate spidroin (Chen et al., 2012) forms typical amyloid-like fibrils, as judged by Thioflavin-T fluorescence, electron microscopy, and Congo red staining. Such fibrils were made by incubating 10 μ M of *A. ventricosus* MiSp domain under quiescent conditions at 25°C in 50 mM sodium acetate buffer, pH 5.2, for 8 h. The fibrils were diluted to 50 nM and studied as described for A β fibrils.

Electron microscopy. Aliquots of 2 μ l were taken from the different A β ₁₋₄₂ preparations, adsorbed on copper grids and negatively stained with 2.5% uranyl acetate in 50% ethanol. Mixed A β ₁₋₄₂ sample (non-SEC purified) stored at -20°C was thawed on ice before being adsorbed on grid. Monomeric A β ₁₋₄₂ was adsorbed on a grid directly after SEC purification and after fibrillization. The samples were examined and photographed using a Hitachi H7100 microscope operated at 75 kV.

Results

Effects of A β on hippocampal gamma oscillations

Previously we have shown that synthetic A β ₁₋₄₂ (A β hereafter) reduces hippocampal gamma oscillations (Nerelius et al., 2009) and septal theta synchronization (Leão et al., 2012). Using high-purity recombinant A β (Walsh et al., 2009) we first characterized the concentration and incubation time-dependence of the A β -

← and decreases IPSC charge transfer. These changes are based on an A β -induced increase of EPSC frequency and amplitude and a decrease of IPSC frequency and amplitude.

Figure 4. Severity of A β effect increases with fibrillization. **A**, Electron micrographs of A β_{1-42} preparations negatively stained with uranyl acetate. Monomeric: in the SEC-purified A β monomer, no aggregated material was found. Mixed: the non-SEC purified A β contained aggregated material but also scarce fibrils. Fibrillar: after incubation of monomeric SEC-purified A β at 37°C, fibrils were abundant. **B**, Example traces and example power spectra of control gamma oscillations and their degradation by monomeric, mixed, and fibrillar A β . **C**, Summary box plot of gamma oscillation degradation by monomeric, mixed, and fibrillar A β .

induced degradation of hippocampal gamma oscillations. Horizontal hippocampal slices from C57BL/6 mice were incubated with varying A β concentrations for varying lengths of time. Subsequent to incubation, gamma oscillations were induced by superfusing slices with 100 nM kainate (KA). LFP recordings in area CA3 revealed control gamma oscillations (no prior incubation with A β) of $1.24 \times 10^{-9} \pm 1.31 \times 10^{-10}$ V 2 power ($n = 19$; Fig. 1; Table 1a).

Keeping the incubation time constant at 180 min we proceeded to test the effect of varying concentrations of A β (mixed A β = containing a mix of all A β conformations). Although incubation with the very low concentration of 2 nM A β (subphysiological for CNS; Sjögren et al., 2001; for review, see Frankfort et al., 2008) resulted in a nonsignificant reduction of gamma oscillation power (Table 1b), a physiological concentration of 50 nM led to a significant reduction (to 20% of control; Table 1c; Fig. 1A,B). Further increase in A β concentration resulted in further gamma power reduction (500 nM A β : to 14% of control; 1 μ M A β : to 1.6% of control; Table 1d,e; Fig. 1A,B). The EDTA-containing solution A β was dissolved in had no effect on hippocampal gamma oscillations in the absence of A β (Table 1f).

In a parallel set of experiments, we kept the A β concentration constant at 1 μ M and varied the incubation time from 15 to 180 min. Even a short A β application time of 15 min resulted in a very strong reduction of gamma oscillation power (to 11% of control;

Table 1g; Fig. 1D). Progressively longer application times resulted in further gamma power reduction (30 min: to 11% of control; 60 min: to 4% of control; 120 min: to 1.7% of control; 180 min: to 1.6% of control; Table 1c,h-j; Fig. 1D).

Finally, we confirmed that the physiological A β concentration of 50 nM yields a significant and acute reduction of gamma oscillation power after only 15 min of incubation (to 55% of control; Table 1k; Fig. 1C,D, inset), which could not be washed-out by superfusion with 100 nM KA solution for 180 min (Table 1l). Because of its physiologically relevant concentration and acute effect, the “50 nM A β for 15 min” paradigm is well suited for the investigation of cellular and network mechanisms underlying the acute A β -induced degradation of gamma oscillations and was used for all subsequent LFP experiments (interface recording chamber for LFP and patch-clamp experiments in submerged recording chamber 1 μ M A β was used to offset the method-dependent lower-signal amplitude in submerged conditions).

To control for potential unspecific peptide effects and ascertain sequence-specificity of the primary agent, A β , we performed experiments with synthetic sequence-inverted A β_{42-1} . To control for possible different effects/efficacy of our recombinantly derived A β versus the synthetic sequence-inverted A β we also performed a control experiment with synthetic A β_{1-42} . Synthetic A β (50 nM, 15 min incubation) resulted in a significant reduction of KA-induced gamma oscillation power to 43% (KA: $5.73 \times 10^{-9} \pm$

Figure 5. Prevention of gamma degradation by interference with A β folding and aggregation. **A**, Example traces and example power spectra of control gamma oscillations and prevention of their degradation by A β in the presence of a designed ligand. **B**, Summary box plot of prevention of gamma oscillation degradation by monomeric, mixed, and fibrillar A β in the presence of a designed ligand. **C**, Example traces and example power spectra of control gamma oscillations and prevention of their degradation by A β in the presence of a chaperone. **D**, Summary box plot of prevention of gamma oscillation degradation by monomeric, mixed, and fibrillar A β in the presence of a BRICHOS chaperone.

$1.75\text{E-}10\text{ V}^2$; $n = 8$; A β : $2.40\text{E-}09 \pm 4.85\text{E-}10\text{ V}^2$; $n = 8$; Mann–Whitney U test: $U = 64$, $p = 0.00016$). The power reduction caused by synthetic A β is not significantly different from the power reduction caused by recombinantly derived A β (both 50 nM, 15 min incubation; Mann–Whitney U test: $U = 60$, $p = 0.384$). In contrast, synthetic sequence-inverted A β (50 nM, 15 min incubation) failed to reduce the power of KA-induced gamma oscillation (to 102%; KA: $6.90\text{E-}09 \pm 9.91\text{E-}10\text{ V}^2$; $n = 8$; A β : $7.03\text{E-}09 \pm 1.96\text{E-}10\text{ V}^2$; $n = 8$; Mann–Whitney U test: $U = 40$, $p = 0.44$). Therefore the described A β -induced effects on gamma oscillations are sequence-specific to A β_{1-42} .

To underscore the relevance of our *in vitro* model for cognition and gamma oscillations *in vivo*, we also tested a second mechanism of gamma oscillation induction *in vitro* for possible A β effects: gamma oscillations were induced by 20 μ M carbachol in the absence of A β to establish control values for gamma oscillation power ($9.86\text{E-}09 \pm 3.32\text{E-}10\text{ V}^2$; $n = 7$). Other slices were incubated for 15 min in an A β solution (50 nM; same protocol as for KA-induced gamma oscillations) before induction of gamma oscillations by 20 μ M carbachol ($3.32\text{E-}09 \pm 2.40\text{E-}10\text{ V}^2$; $n = 7$). Our data show that carbachol-induced gamma oscillations are significantly reduced to 34% of control by A β (Mann–Whitney U test: $U = 49$, $p = 0.0006$) and that the described A β -induced reduction of gamma oscillations is not specific to kainate-induced gamma oscillations.

A β desynchronizes generation of pyramidal cell APs

Desynchronization of AP firing in various cell types is a possible cause for degradation of network rhythms (Andersson et al.,

2010, 2012; Leão et al., 2012). Recording pyramidal cell (PC) AP in area CA3 in cell-attached patch-clamp mode and concomitant extracellular gamma oscillations (100 nM KA) we found that application of A β (1 μ M) led to a steady degradation of gamma oscillation power (to $80 \pm 2.3\%$ of control, $n = 26$, $p < 0.0001$; Fig. 2A) while leaving gamma oscillation frequency unchanged (control: 31.05 ± 0.95 Hz; A β : 31.59 ± 1.17 Hz; $n = 26$, $p = 0.720$; Fig. 2B). Concomitantly to the extracellular changes A β led to an increase in AP firing in PCs (to $159 \pm 26\%$ of control, $n = 12$, $p < 0.05$; control: 3.50 ± 0.63 Hz; A β : 4.90 ± 0.71 Hz; Fig. 2C). Analysis showed that although the mean phase of AP firing stayed the same (δ AP phase = 0, $n = 26$; Fig. 2D), the firing window became wider consistent with a desynchronization of PCs (AP phase Gaussian fit half-width $211 \pm 31\%$ of control, $n = 12$, $p < 0.005$; Fig. 2E, F). Note that both degradation of gamma oscillation power, increase of PC AP firing and increase of AP phase Gaussian half-width follow the same kinetics and plateau 10–15 min after A β application.

A β alters excitation/inhibition balance independently from network activation state

Gamma oscillations depend on a tightly regulated equilibrium between inhibition and excitation in the neuronal network. Alterations to this balance are a possible cause to altered network rhythms. We therefore investigated the effect of A β (1 μ M) on excitatory and IPSCs in CA3 PCs. EPSCs ($V_h = -70$ mV) and IPSCs ($V_h = 0$ mV) were recorded from PCs in an activated neuronal network (100 nM KA present). Analysis showed that EPSC charge transfer increased in the presence of A β (to $123 \pm$

6% of control, $n = 6$, $p < 0.05$), whereas IPSC charge transfer decreased (to $72 \pm 6.7\%$ of control, $n = 6$, $p < 0.05$; Fig. 3A). Changes to postsynaptic current charge transfer can be caused by either changes in current frequency and/or amplitude. Our experiments showed that A β caused an increase of both EPSC frequency (control: 19.15 ± 0.25 Hz; A β : to $116 \pm 5\%$ of control, $n = 6$, $p < 0.05$) and amplitude (control: 51.04 ± 0.21 pA; A β : to $115 \pm 3\%$ of control, $n = 6$, $p < 0.001$; Fig. 3A), while causing a decrease of both IPSC frequency (control: 42.67 ± 0.09 Hz; A β : to $73 \pm 10\%$ of control, $n = 6$, $p < 0.05$) and amplitude (control: 124.21 ± 0.35 pA; A β : to $68 \pm 10\%$ of control, $n = 6$, $p < 0.05$; Fig. 3A).

To ascertain that the A β -induced alteration of excitatory/inhibitory balance is not dependent on the activation state of the neuronal network, we repeated our EPSC/IPSC recordings in quiescent slices (no KA present). Both spontaneous EPSCs and spontaneous IPSCs recorded under quiescent conditions showed the same response to A β as postsynaptic currents in the activated network. Spontaneous EPSC charge transfer increased to $129 \pm 7.6\%$ of control ($n = 13$, $p < 0.005$) with both frequency and amplitude increasing (control frequency: 24.86 ± 0.09 Hz; A β frequency: to $122 \pm 7.7\%$ of control; control amplitude: 17.00 ± 0.14 pA; A β amplitude: to $111 \pm 4.5\%$ of control; both $p < 0.05$, both $n = 13$; Fig. 3B). Spontaneous IPSC charge transfer decreased to $71 \pm 5.1\%$ of control ($n = 14$, $p < 0.0001$) with both frequency and amplitude decreasing (control frequency: 33.54 ± 0.12 Hz; A β frequency: to $81 \pm 5\%$ of control, $p < 0.001$; control amplitude: 14.93 ± 0.07 pA; A β amplitude: to $76 \pm 4.5\%$ of control, $p < 0.0005$; both $n = 14$; Fig. 3B).

Consistent with the above results cumulative probability distributions analysis showed that amplitudes of EPSCs increase moderately while their inter-event-intervals decrease in both the activated and the quiescent network state. In contrast, the amplitudes of IPSCs decrease and their IEs increase in both the activated and the quiescent network state. Finally, we conducted paired-pulse experiments in CA3 pyramidal cells (no KA present) with mossy-fiber pathway stimulation. The ratio between the first to second EPSP slope decreased from 1.082 ± 0.109 in control conditions to 0.872 ± 0.1119 after 20 min exposure to $1 \mu\text{M}$ A β ($n = 65$, $p < 0.01$). This suggests that A β modifies presynaptic release dynamics, which may contribute to the observed increase in EPSC amplitude.

Severity of A β effect increases with fibrillization

Knowledge of which step in the misfolding and aggregation sequence of A β is the (most) cytotoxic and responsible for the cellular and network changes seen in AD remains sparse and contradictory. Arguments have been advanced for soluble A β oligomers rather than fully formed A β fibrils (Klein et al., 2001;

Figure 6. Preservation of excitatory/inhibitory balance by interference with A β folding and aggregation. **A**, In an activated network (100 nM KA), a designed ligand prevents the A β -induced increase in EPSC charge transfer and decrease in IPSC charge transfer. **B**, In an activated network (100 nM KA), a BRICHOS chaperone prevents the A β -induced increase in EPSC charge transfer and decrease in IPSC charge transfer.

Nimmrich and Ebert, 2009). Using purified A β preparations that contained either monomeric A β , fibrillar A β or a mix of all conformations (mixed A β ; all 50 nM, 15 min incubation; Fig. 4A) we tested their ability to degrade gamma oscillations. Our experiments showed that with increasing presence of fibrillized A β forms in the incubation solution the level of gamma oscillation degradation also increases when compared with controls (Table 1m): monomeric A β : to 66% of control; mixed A β : to 35% of control; fibrillar A β : to 19% of control; Table 1n–p; Figure 4B,C). The results of this experiment suggest that the fibrillar form of A β is the most effective in eliciting detrimental network and cellular effects. Because it has been shown that secondary nucleation into oligomeric species is mediated by A β monomers that bind to the surface of fibrils, and that this is a major source of toxic species (Cohen et al., 2013) we conclude that fibrillar A β may be toxic as such, but may also cause toxicity by nucleating the formation of oligomers.

To exclude the possibility that the strong degradation of gamma oscillations we see in the presence of fully fibrillized A β is due to unspecific fibril effects, we conducted a control experiment in which fibrils derived from a spider silk protein domain were substituted for A β fibrils. Incubating hippocampal slices

Table 1. Summary of raw data values, *n* numbers, and statistical data for all experiments

	Condition	Power (V^2)	<i>n</i>	Mann–Whitney <i>U</i> test			
				<i>U</i>	<i>n1</i>	<i>n2</i>	<i>p</i>
a	100 nM KA control series 1	$1.24 \times 10^{-09} \pm 1.31 \times 10^{-10}$	19				
b	2 nM A β , 180 min	$8.26 \times 10^{-10} \pm 1.66 \times 10^{-10}$	8	106	19	8	0.119
c	50 nM A β , 180 min	$2.54 \times 10^{-10} \pm 8.28 \times 10^{-11}$	8	143	19	8	<0.0001
d	500 nM A β , 180 min	$1.79 \times 10^{-10} \pm 6.16 \times 10^{-11}$	8	146	19	9	<0.0001
e	1 μ M A β , 180 min	$1.95 \times 10^{-11} \pm 9.55 \times 10^{-12}$	8	114	19	6	<0.0001
f	1 μ M EDTA solution, 180 min	$1.99 \times 10^{-09} \pm 1.01 \times 10^{-09}$	7	69	19	7	0.91
g	1 μ M A β , 15 min	$1.38 \times 10^{-10} \pm 5.11 \times 10^{-11}$	9	168	19	9	<0.0001
h	1 μ M A β , 30 min	$1.38 \times 10^{-10} \pm 5.19 \times 10^{-11}$	10	185	19	10	<0.0001
i	1 μ M A β , 60 min	$5.53 \times 10^{-11} \pm 1.56 \times 10^{-11}$	9	170.5	19	9	<0.0001
j	1 μ M A β , 120 min	$2.21 \times 10^{-11} \pm 6.05 \times 10^{-12}$	15	285	19	15	<0.0001
k	50 nM A β , 15 min	$6.86 \times 10^{-10} \pm 1.68 \times 10^{-10}$	8	119.5	19	8	0.019
l	50 nM A β , 15 min + 180 min washout	$8.20 \times 10^{-10} \pm 2.32 \times 10^{-10}$	7	25	7	7	1
m	100 nM KA control series 2	$5.58 \times 10^{-09} \pm 3.98 \times 10^{-10}$	16				
n	50 nM Monomeric A β , 15 min	$3.71 \times 10^{-09} \pm 5.59 \times 10^{-10}$	14	167	16	14	0.022
o	50 nM Mixed A β , 15 min	$1.97 \times 10^{-09} \pm 2.98 \times 10^{-10}$	12	130	14	12	0.017
p	50 nM Fibrillar A β , 15 min	$1.08 \times 10^{-09} \pm 1.11 \times 10^{-10}$	12	107	12	12	0.04
q	50 nM Spider silk fibrils, 15 min	$6.27 \times 10^{-09} \pm 2.03 \times 10^{-10}$	6	56	16	6	0.59
r	50 nM Spider silk fibrils, 180 min	$5.19 \times 10^{-09} \pm 1.85 \times 10^{-10}$	5	46	16	5	0.66
s	250 nM Dec-DETA + 50 nM monomeric A β	$7.68 \times 10^{-09} \pm 6.22 \times 10^{-10}$	8	85	16	8	0.214
t	250 nM Dec-DETA + 50 nM mixed A β	$4.94 \times 10^{-09} \pm 4.08 \times 10^{-10}$	14	134	16	14	0.377
u	250 nM Dec-DETA + 50 nM fibrillar A β	$3.55 \times 10^{-09} \pm 3.42 \times 10^{-10}$	8	109	16	8	0.0045
v	250 nM Dec-DETA	$5.55 \times 10^{-09} \pm 5.23 \times 10^{-10}$	8	64.5	16	8	0.976
w	1 μ M BRICHOS + 50 nM monomeric A β	$5.06 \times 10^{-09} \pm 3.23 \times 10^{-10}$	15	144.5	16	15	0.337
x	1 μ M BRICHOS + 50 nM mixed A β	$5.43 \times 10^{-09} \pm 3.49 \times 10^{-10}$	8	71	16	8	0.697
y	1 μ M BRICHOS + 50 nM fibrillar A β	$2.72 \times 10^{-09} \pm 3.54 \times 10^{-10}$	8	121	16	8	0.00012
z	1 μ M BRICHOS	$5.22 \times 10^{-09} \pm 1.07 \times 10^{-10}$	8	77	16	8	0.452

with 50 nM spider silk-derived fibrils for 15 min as well as 180 min did not alter the size of subsequently induced gamma oscillations (Table 1q,r). We conclude that the strong degradation of gamma oscillations by A β fibrils is A β -specific and not due to unspecific fibril effects.

Prevention of gamma degradation by interference with A β -folding and aggregation

Several compounds are currently available that can interfere with the A β -folding and aggregation sequence at different points (Schenk et al., 2012). We used two such compounds to further determine the (most) cytotoxic conformation of A β . Dec-DETA is a designed ligand that shifts the equilibrium between the α -helical and the β -sheet secondary peptide conformation of A β toward the α -helical form (Nerelius et al., 2009; Ito et al., 2012). Because the β -sheet form is necessary for both potential pore formation (Jang et al., 2010; Sepulveda et al., 2010) as well as fibril formation, Dec-DETA limits both by interfering at a very early point in A β 's folding and aggregation sequence. When incubating slices for 15 min with monomeric A β , mixed A β , or fibrillar A β (all 50 nM) in the presence of Dec-DETA (250 nM), we found that the presence of the ligand completely prevented the degradation of gamma oscillations by monomeric A β (Table 1s) and mixed A β (Table 1t), and partially prevented degradation by fibrillar A β (to 63% of control; Table 1u) (Fig. 5A,B). Control experiments with Dec-DETA-only incubation showed no change of gamma oscillation power (Table 1v) compared with control gamma oscillations.

The second compound used was recombinant human BRICHOS domain from ProSPC, which delays aggregation of A β into β -sheet oligomers and fibrils *in vitro* (Willander et al., 2012; Knight et al., 2013) and *in vivo* (Hermansson et al., 2014). Compared with Dec-DETA ProSPC BRICHOS limits fibril formation by interfering at a later point in A β 's folding and aggregation sequence. When incubating slices for 15 min with monomeric

A β , mixed A β , or fibrillar A β (all 50 nM) in the presence of ProSPC BRICHOS (1 μ M), we again found that the presence of the ligand completely prevented the degradation of gamma oscillations by monomeric A β (Table 1w) and mixed A β (Table 1x), and partially prevented degradation by fibrillar A β (to 48% of control; Table 1y; Fig. 5C,D). Control experiments with ProSPC BRICHOS-only incubation showed no change of gamma oscillation power (Table 1z) compared with control gamma oscillations.

It is interesting to note that the effectiveness of both Dec-DETA as well as ProSPC BRICHOS to prevent degradation of gamma oscillations follows the effectiveness of the particular A β solution to induce it (in the presence of Dec-DETA: monomeric A β to mixed A β : $U = 85.5$, $n1 = 14$, $n2 = 8$, $p = 0.042$; mixed A β to fibrillar A β : $U = 87.0$, $n1 = 14$, $n2 = 8$, $p = 0.035$; in the presence of ProSPC BRICHOS: monomeric A β to mixed A β : $U = 82.0$, $n1 = 15$, $n2 = 8$, $p = 0.169$; mixed A β to fibrillar A β : $U = 63.0$, $n1 = 8$, $n2 = 8$, $p = 0.00031$; Fig. 5B,D).

Preservation of excitatory/inhibitory balance by interference with A β folding and aggregation

We have shown that designed ligands that interfere with A β -folding (Dec-DETA) and aggregation (ProSPC BRICHOS) prevent the A β -induced degradation of gamma oscillations. Because A β also alters the balance between excitation and inhibition on the cellular level, we proceeded to test whether Dec-DETA and ProSPC BRICHOS act to protect this balance against A β -induced changes, explaining their protective effect on extracellular gamma oscillations. We therefore investigated the effect of A β (1 μ M) in the presence of either ligand on inhibitory and EPSCs in CA3 PC. EPSCs ($V_h = -70$ mV) and IPSCs ($V_h = 0$ mV) were recorded from PCs in an activated neuronal network (100 nM KA present). Analysis showed that both EPSC and IPSC charge transfer remained unaltered in the presence of A β and Dec-DETA (5 μ M; EPSCs: $95 \pm 4\%$ of control, $n = 5$, $p > 0.37$; IPSCs: $97 \pm 4\%$ of control, $n = 5$, $p < 0.49$; Fig. 6A). Likewise, in the presence of

ProSPC BRICHOS (1 μ M) EPSC and IPSC charge transfer remained unaltered (EPSCs: $99 \pm 3\%$ of control, $n = 5$, $p > 0.69$; IPSCs: $102 \pm 7\%$ of control, $n = 4$, $p < 0.79$; Fig. 6B). We conclude that the protective effect of Dec-DETA and ProSPC BRICHOS on gamma oscillation power is founded on the compounds ability to prevent the A β -induced alteration of the excitatory/inhibitory balance in the hippocampal network.

Discussion

In this study, we investigated the cellular mechanisms responsible for the degrading effect of A β on AD-relevant functional network dynamics, namely gamma oscillations, as well as means to prevent the neurotoxic effect of A β . By doing so we have explored the functional link between the main culprit for the cognitive deficits observed in AD patients, A β , and a brain rhythm that underlies higher cognitive functions and is known to be significantly degraded in AD patients, who at the same time suffer from deficiencies in their cognitive faculties (Ribary et al., 1991; Uhlhaas and Singer, 2006; Jelic and Kowalski, 2009). Furthermore, we have linked the level of A β toxicity to the initially available concentration of fibrillar over monomeric A β forms and have demonstrated that compounds that interfere with A β aggregation can prevent A β neurotoxicity.

Gamma oscillations are a type of brain rhythm that is particularly important for higher brain functions, such as cognition, attention, and memory (Singer, 1993). As a rhythmic electrical brain activity its generation and maintenance is dependent on the synchronization of action potential firing of different cell classes and the tightly regulated balance of excitation and inhibition in the neuronal circuitry. It is therefore only consistent that the degrading effect of A β on gamma oscillation strength is caused, as our data show, by an A β -induced desynchronization of AP firing in pyramidal cells and a general shift of the balance between excitation and inhibition in the hippocampal circuitry.

Our data show an increasing A β -induced degradation of gamma oscillations with increasing concentration of initially available fibrillar over monomeric A β forms. Importantly, most of the degrading effect on gamma oscillations is caused by nanomolar concentrations of A β , which is comparable to physiological A β concentrations, and contrasts favorably with the concentrations in the micromolar range used in many other studies. Likewise, the designed ligand Dec-DETA and the chaperone domain BRICHOS from ProSPC used to prevent A β toxicity demonstrate a decreasing prevention effectiveness with increasing concentration of initially available fibrillar over monomeric A β forms. Although both results overtly point to the fibrillar form of A β as the major source of the peptides toxicity, and as such, appear contrary to the oligomeric hypothesis of AD this is not necessarily the case: arguably it is possible that A β fibrils have some inherent toxicity. However, given their sheer size it seems unlikely that they are able to penetrate our hippocampal slice preparation readily enough to explain a profound degradation of cellular parameters and gamma oscillations after only 15 min of exposure. Penetration of single cells seems even less likely. However, it is plausible that a higher initial concentration of fibrillar A β translates into a higher concentration of oligomeric A β because of the deconstruction of fibrils into oligomers and the induction of oligomer production by secondary nucleation (Cohen et al., 2013).

Our study provides correlative evidence for a link between A β -induced effects on synaptic currents and AD-relevant neuronal network oscillations. Our findings of the coupling of A β toxicity to initially available concentration of fibrillar over

monomeric A β forms, together with other recent findings on A β secondary nucleation mechanisms (Cohen et al., 2013), open the door to combine research obtained on the molecular A β level with functional electrophysiological investigations in AD-relevant brain activity. Finally, our study underscores the promising approach to prevent A β toxicity by stabilizing the peptide's α -helical form (Nerelius et al., 2009) or preventing its aggregation into fibrillar form (Knight et al., 2013; Hermansson et al., 2014) by providing evidence that these potentially therapeutic approaches indeed prevent the underlying cellular causes of A β toxicity for functional network dynamics, namely action potential desynchronization and shift of excitatory/inhibitory balance in the neuronal network.

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